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# Arterial amino acid concentrations in sheep consuming forage diets

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**Abstract**

Arterial blood collected from sheep was analyzed for various amino acids to evaluate effects of dietary forage characteristics on adequacy of amino acid availability for protein synthesis. In three experiments with grass diets consumed ad libitum or in limited quantities by nearly mature wethers, differences in grass source and(or) quality generally did not markedly affect arterial concentrations of essential amino acids. In the fourth experiment, wethers (7.5 months of age and $31 \pm 0.8$ kg BW) consumed ad libitum bermudagrass (*Cynodon dactylon*; BG) and ryegrass (*Lolium multiflorum*)–wheat (*Triticum aestivum*; RW) hay mixtures in percentages of 0, 33, 67 and 100%. As DE intake decreased with increasing dietary level of BG, arterial lysine concentration increased linearly ($P = 0.02$; 116, 137, 144 and 157 $\mu$M for 0, 33, 67 and 100% BG, respectively); whereas, concentrations of other essential amino acids did not differ among treatments. In the fifth experiment, wethers (8.5 months of age and $33 \pm 0.9$ kg BW) consumed ad libitum BG or RW either coarsely chopped or finely ground and pelleted; DE intake was greater for RW vs. BG and for pelleted than for chopped grass. Lysine concentration was greater ($P = 0.01$) for BG than for RW and was decreased ($P = 0.03$) by pelleting (133, 118, 114 and 78 $\mu$M for chopped BG, pelleted BG, chopped RW and pelleted RW, respectively). Conversely, concentrations of tryptophan, valine, phenylalanine, isoleucine and leucine were greater ($P < 0.03$) for RW than for BG, and grinding and pelleting increased concentrations of valine ($P = 0.07$) and phenylalanine ($P = 0.06$). In conclusion, DE intake with grass diets may influence particular amino acids most limiting to protein synthesis by growing ruminants, with lysine availability being of relatively greater concern with forages yielding high vs. low DE intake. 

*Keywords:* Sheep; Amino acids; Forage

## 1. Introduction

Most amino acids absorbed by ruminants ingesting forage diets are of microbial origin, in part because of generally extensive microbial degradation of forage proteins (ARC, 1980; Minson, 1990). The most important determinant of microbial protein production is the quantity of ruminally fermentable OM, with forage intake having greatest influence on yield of microbial protein (ARC, 1980). Ruminant amino acid requirements are markedly influenced by stage

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of maturity and growth rate (Owens and Bergen, 1983). In turn, growth rate is affected by ME intake (ARC, 1980). Potential growth rate and rate of protein synthesis influence the total quantity of amino acids that can be incorporated in synthesized protein and also alter the array of specific amino acids required (Owens and Bergen, 1983). Thus, characteristics of forage-based diets affect both amino acid absorption and their potential utilization in protein synthesis. Hence, the degree to which specific amino acids limit protein synthesis might vary with attributes or composition of forage-based diets through effects on absorption of specific amino acids and ME intake.

Arterial or jugular concentrations of amino acids have been used to provide information regarding relationships between availabilities and requirements for maximal protein synthesis (Nimrick et al., 1970a,b; Fenderson and Bergen, 1975; Gibb et al., 1992). Besides absorption and rate of incorporation in synthesized protein, blood concentrations of essential amino acids are affected by tissue release and catabolism, and free amino acids in blood represent a small proportion of the whole-body protein–amino acid pool (Owens and Bergen, 1983). Nonetheless, it is generally accepted that an increase in arterial amino acid concentration resulting from dietary change indicates increased amino acid availability relative to potential for use in protein synthesis (Egan and Rogers, 1978). Conversely, a decrease in arterial amino acid concentration implies the opposite change, depending on extent of metabolism in alternate functions (e.g., oxidation, gluconeogenesis).

Objectives of this experiment were to determine the effects of various dietary forage treatments on arterial concentrations of most essential amino acids, thereby to provide insight into adequacy of amino acid availability relative to need for, or potential for use in, protein synthesis. Specific effects investigated were of dietary (1) grass sources differing in quality with ad libitum consumption by nearly mature sheep; (2) grass source and legume level with ad libitum consumption by nearly mature sheep; (3) grass source with a restricted level of feed intake by nearly mature sheep; (4) proportions of different grass sources with ad libitum consumption by growing sheep; and (5) grass source and physical form with ad libitum consumption by growing sheep.

## 2. Materials and methods

Arterial whole blood and forage samples from five experiments were analyzed for amino acids. In Experiment 1 (Patil et al., 1995b), wethers (approximately 18 months old and $44 \pm 0.7$ kg BW) consumed ad libitum coarsely chopped bermudagrass (*Cynodon dactylon*; 24- and 42-day regrowth and full-season growth), endophyte-free fescue (*Festuca arundinacea*; early head emergence) or orchardgrass (*Dactylis glomerata*; post-anthesis and with seed in dough stage) hay. In Experiment 2 (Patil et al., 1996), wethers (approximately 20 months of age and $45 \pm 0.7$ kg BW) consumed ad libitum coarsely chopped bermudagrass (vegetative growth stage) or ryegrass (*Lolium multiflorum*; late-vegetative to boot stage)–wheat (*Triticum aestivum*; post-anthesis to early milk stage) hay with 0, 20 or 40% alfalfa (*Medicago sativa*; full-bloom to early seed pod stage) hay. In Experiment 3 (Patil et al., 1995a), wethers (approximately 22 months of age and $46 \pm 1.3$ kg BW) consumed the same bermudagrass and ryegrass–wheat hay sources used in Experiment 2 at 1.6% of BW (DM basis). In Experiment 4 (Goetsch et al., 1997b), wethers (approximately 7.5 months of age and $31 \pm 0.8$ kg BW) consumed ad libitum coarsely chopped bermudagrass (vegetative growth stage), ryegrass (late-vegetative to boot stage)–wheat (post-anthesis to early milk stage) hay or mixtures thereof (33 and 67% bermudagrass and 67 and 33% ryegrass–wheat, respectively; air–dry basis). In Experiment 5 (Goetsch et al., 1997a), wethers (approximately 8.5 months old and $33 \pm 0.9$ kg BW) consumed ad libitum the same bermudagrass and ryegrass–wheat hay sources used in Experiment 4 in coarsely chopped or finely ground and pelleted form.

In Experiments 1, 2 and 3, sheep received two meals daily, with a 12-h feeding interval, and in Experiments 4 and 5, an 8-h feeding interval was employed, with three meals daily. Experiments or periods were 21 days, with blood sampling on one of the last few days. Blood samples in Experiments 1, 2 and 3 were taken hourly to represent $-2$, $-1$, 0, 1, 2, 3, 4, 5 and 6 h after feeding. In Experiments 4 and 5, samples were obtained at each hour of the 8-h feeding interval. Whole blood samples were collected in tubes that contained potassium oxalate and sodium fluoride and placed in ice. Composite sam-

ples were formed from individual hourly samples on a volume basis, deproteinized with sulfosalicylic acid (Harmon et al., 1991) and stored frozen at −80°C. Samples were later analyzed in triplicate for amino acids by reversed-phase high performance liquid chromatography following pre-column derivatization with *ortho*-phthalaldehyde (Microsorb™ Type O AAAnalysis column and guard module; Rainin, 1990), using L-homoserine as an internal standard (1.85 μM).

Data were statistically analyzed as described by Patil et al. (1995b) (Experiment 1), Patil et al. (1996) (Experiment 2), Patil et al. (1995a) (Experiment 3), Goetsch et al. (1997a) (Experiment 4) and Goetsch et al. (1997b) (Experiment 5). Simple correlation coefficients between arterial concentrations of some amino acids and other variables (e.g., digestible N intake) were determined (SAS, 1990). For arterial concentrations of some amino acids, other variables were included in the model singly as a covariate preceding other sources of variation. Probabilities for Type I sums-of-squares were examined to aid in ascertaining if treatment effects on arterial amino acid concentrations were because of effects of covariates.

## 3. Results

### 3.1. Experiment 1

Forage CP concentration was 17.1, 15.6, 12.9, 12.7, 10.8 and 5.4% (DM basis), DM intake was 1.05, 1.00, 1.11, 1.00, 0.96 and 0.60 kg day$^{-1}$ (SE 0.053), DE intake was 11.9, 10.8, 11.3, 12.4, 10.9 and 6.3 MJ day$^{-1}$ (SE 0.657) and digestible N intake was 19.9, 16.4, 14.4, 14.0, 9.3 and 2.0 g day$^{-1}$ (SE 0.76) for high, moderate and low quality tropical grasses and for high, moderate and low quality temperate grasses, respectively (Patil et al., 1995b).

Treatment differences in arterial whole blood amino acid concentrations were observed for four of the 15 amino acids measured, three of which were essential (Table 1). Serine concentration in arterial blood was greater ($P < 0.05$) for low quality temperate grass than for high quality tropical and temperate grasses. Overall, with both grass sources, arterial concentrations of valine, isoleucine and leucine were greatest among qualities for moderate quality grasses. Main effect means of valine and isoleucine concen-

Table 1
Arterial whole blood amino acid concentrations (μM) in sheep consuming ad libitum different qualities of tropical and temperate grass (Experiment 1)

| Amino acid | Tropical grass[a] | | | Temperate grass[a] | | | SE | Grass source $P$ (≤) |
|---|---|---|---|---|---|---|---|---|
| | H | M | L | H | M | L | | |
| Aspartate | 24.8 | 11.0 | 21.0 | 24.4 | 10.5 | 28.7 | 6.61 | |
| Glutamate | 83.5 | 48.9 | 88.3 | 59.3 | 74.4 | 103.6 | 8.87 | |
| Asparagine | 60.5 | 72.2 | 78.3 | 58.7 | 74.4 | 59.0 | 7.54 | |
| Serine | 64.2[b] | 91.3[b,c] | 99.0[b,c] | 68.3[b] | 95.1[b,c] | 104.8[c] | 10.31 | |
| Glutamine | 172 | 313 | 262 | 259 | 258 | 324 | 38.2 | |
| Alanine | 59.7 | 118.7 | 115.8 | 86.8 | 108.0 | 147.0 | 19.73 | |
| Tyrosine | 88.1 | 98.8 | 77.4 | 74.1 | 101.9 | 70.6 | 20.30 | |
| Ornithine | 152.6 | 176.5 | 147.4 | 88.4 | 116.0 | 115.8 | 19.32 | |
| Histidine | 43.7 | 68.1 | 45.4 | 62.5 | 71.1 | 72.7 | 18.85 | |
| Tryptophan | 25.3 | 30.8 | 26.2 | 21.2 | 19.4 | 17.1 | 4.49 | |
| Valine | 190[b,c] | 252[c] | 167[b] | 147[b] | 195[b,c] | 140[b] | 25.8 | 0.08 |
| Phenylalanine | 45.3 | 56.0 | 43.3 | 39.9 | 44.0 | 39.5 | 4.54 | |
| Isoleucine | 70.5[b,c,d] | 86.4[d] | 65.0[b,c,d] | 46.1[a] | 75.6[c,d] | 57.2[b,c] | 8.83 | 0.08 |
| Leucine | 104.8[b,c] | 142.4[c] | 96.2[b] | 90.7[b] | 113.3[b,c] | 98.0[b] | 12.25 | |
| Lysine | 109.6 | 129.1 | 113.6 | 95.2 | 114.2 | 138.0 | 11.27 | |

[a] H = highest quality; M = moderate quality; L = lowest quality.
[b,c,d] Means in a row without a common superscript differ ($P < 0.05$).

Table 2
Arterial whole blood amino acid concentrations ($\mu$M) in sheep consuming bermudagrass or ryegrass–wheat and different levels of alfalfa (A; Experiment 2)

| Amino acid | Bermudagrass | | | Ryegrass–wheat | | | SE | $P$ ($\leq$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0% A | 20% A | 40% A | 0% A | 20% A | 40% A | | Grass source | Alfalfa inclusion | Alfalfa level |
| Aspartate | 18.8 | 16.9 | 23.5 | 19.5 | 20.3 | 20.5 | 2.27 | | | 0.05 |
| Glutamate | 59.6 | 55.3 | 60.0 | 65.5 | 69.3 | 68.5 | 3.62 | | | |
| Asparagine | 47.1 | 46.9 | 49.5 | 47.1 | 46.6 | 53.4 | 3.10 | | | |
| Serine | 52.8 | 40.4 | 52.6 | 41.7 | 48.7 | 52.8 | 5.81 | | | |
| Glutamine | 268 | 268 | 318 | 237 | 227 | 238 | 21.0 | 0.06 | | |
| Alanine | 96.5 | 91.5 | 123.9 | 99.4 | 109.9 | 118.9 | 7.87 | | 0.08 | 0.05 |
| Tyrosine | 67.9 | 72.1 | 77.0 | 67.7 | 81.8 | 78.3 | 11.06 | | | |
| Ornithine | 92.0 | 88.0 | 108.0 | 102.3 | 115.1 | 137.0 | 11.08 | | | 0.10 |
| Histidine | 52.2 | 53.0 | 64.4 | 55.1 | 52.1 | 61.5 | 3.93 | | | 0.03 |
| Tryptophan | 43.2 | 41.4 | 43.4 | 31.2 | 44.8 | 42.8 | 5.27 | | | |
| Valine | 115 | 116 | 136 | 110 | 119 | 151 | 10.8 | | 0.08 | 0.05 |
| Phenylalanine | 40.3 | 40.3 | 41.6 | 39.9 | 41.5 | 49.6 | 3.27 | | | |
| Isoleucine | 45.4 | 46.1 | 51.8 | 43.1 | 48.8 | 57.4 | 5.58 | | | |
| Leucine | 86.0 | 90.3 | 111.3 | 74.2 | 79.8 | 101.3 | 6.24 | | 0.01 | 0.01 |
| Lysine | 65.6 | 63.9 | 80.2 | 70.3 | 77.4 | 96.4 | 7.33 | | 0.09 | 0.04 |

trations were lower ($P = 0.08$) for tropical vs. temperate grasses.

### 3.2. Experiment 2

Crude protein concentration was 13.9, 10.1 and 16.9% (DM basis) in bermudagrass, ryegrass–wheat and alfalfa, respectively. Dry matter intake was 1.13, 1.31, 1.41, 1.15, 1.30 and 1.34 kg day$^{-1}$ (SE 0.044), digestible N intake was 17.6, 20.2, 23.4, 10.1, 14.0 and 17.3 g day$^{-1}$ (SE 1.09) and DE intake was 13.5, 14.3, 15.3, 13.5, 14.9 and 15.4 MJ day$^{-1}$ (SE 0.87) for bermudagrass with 0, 20 and 40% alfalfa and for ryegrass–wheat with 0, 20 and 40% alfalfa, respectively (Patil et al., 1996).

Treatment effects on arterial concentrations of four nonessential and four of the seven essential amino acids measured occurred Table 2). Aspartate, ornithine and histidine concentrations were greater ($P = 0.05$, 0.10 and 0.03, respectively) for 40 vs. 20% alfalfa, and glutamine concentration was greater ($P = 0.06$) for bermudagrass than for ryegrass–wheat. Concentrations were increased by alfalfa inclusion and were greater with 40 vs. 20% alfalfa for alanine ($P = 0.08$ and 0.05, respectively), valine ($P = 0.08$ and 0.05, respectively), leucine ($P = 0.01$) and lysine ($P = 0.09$ and 0.04, respectively).

### 3.3. Experiment 3

Crude protein concentration in bermudagrass and ryegrass–wheat was 13.6 and 9.9% (DM basis), respectively. Dry matter intake was 729 and 730 g day$^{-1}$, digestible N intake was 11.5 and 8.0 g day$^{-1}$ (SE 0.13) and DE intake was 8.5 and 9.3 MJ day$^{-1}$

Table 3
Arterial whole blood amino acid concentrations ($\mu$M) in sheep consuming bermudagrass and ryegrass–wheat at 1.6% BW (DM; Experiment 3)

| Amino acid | Bermudagrass | Ryegrass–wheat | SE |
|---|---|---|---|
| Aspartate | 23.9 | 26.1 | 1.94 |
| Glutamate | 75.8 | 68.6 | 6.57 |
| Asparagine | 47.1 | 37.5 | 3.53 |
| Serine | 56.2 | 43.1 | 6.44 |
| Glutamine | 310 | 261 | 36.5 |
| Alanine | 113 | 110 | 8.6 |
| Tyrosine | 46.4 | 44.2 | 3.53 |
| Ornithine | 107 | 101 | 5.9 |
| Histidine | 58.4 | 55.0 | 4.71 |
| Tryptophan | 41.6 | 38.5 | 1.87 |
| Valine | 105.2 | 88.6 | 6.84 |
| Phenylalanine | 36.9 | 34.2 | 2.32 |
| Isoleucine | 39.0 | 32.5 | 3.90 |
| Leucine | 79.8 | 68.3 | 5.61 |
| Lysine | 77.2 | 62.6 | 5.54 |

Table 4
Arterial whole blood amino acid concentrations ($\mu$M) in sheep consuming different proportions of bermudagrass and ryegrass–wheat hay (Experiment 4)

| Amino acid | % Bermudagrass hay | | | | SE |
|---|---|---|---|---|---|
| | 0 | 33 | 67 | 100 | |
| Aspartate | 44.4 | 42.7 | 48.6 | 45.0 | 8.29 |
| Glutamate | 86.4 | 98.9 | 92.5 | 117.0 | 15.11 |
| Asparagine | 67.7 | 66.6 | 70.7 | 74.4 | 7.11 |
| Serine | 70.9 | 88.1 | 82.9 | 85.2 | 12.07 |
| Glutamine | 310 | 367 | 349 | 348 | 34.1 |
| Alanine | 141 | 155 | 175 | 173 | 16.8 |
| Tyrosine | 73.5 | 68.5 | 70.3 | 59.1 | 8.05 |
| Ornithine a | 106 | 157 | 113 | 131 | 19.0 |
| Histidine | 64.1 | 69.8 | 81.7 | 80.8 | 8.32 |
| Tryptophan | 45.7 | 51.6 | 58.5 | 45.7 | 9.50 |
| Valine | 168 | 164 | 190 | 143 | 15.9 |
| Phenylalanine | 46.3 | 44.5 | 45.9 | 40.5 | 3.54 |
| Isoleucine | 86.1 | 82.5 | 77.4 | 73.0 | 8.02 |
| Leucine | 118.0 | 108.4 | 115.8 | 98.0 | 8.64 |
| Lysine b | 116 | 137 | 144 | 157 | 11.3 |

a, $P \leq 0.10$ (Cubic), b, $P \leq 0.02$ (Linear).

(SE 0.13) for bermudagrass and ryegrass–wheat, respectively (Patil et al., 1995a). Arterial amino acid concentrations did not differ ($P > 0.10$) between grass sources (Table 3).

### 3.4. Experiment 4

Bermudagrass and ryegrass–wheat were 8.0 and 12.9% CP (DM basis), respectively. Dry matter intake was 1.03, 0.92 and 0.76 kg day$^{-1}$ (SE 0.056), digestible N intake was 12.8, 8.4, 7.1 and 4.5 g day$^{-1}$ (SE 0.74) and DE intake was 12.2, 9.8, 9.8 and 7.6 MJ day$^{-1}$ for 0, 33, 67 and 100% bermudagrass, respectively (SE 0.73; Goetsch et al., 1997a). Arterial concentrations of ornithine (cubic; $P = 0.10$) and lysine (linear; $P = 0.02$) were affected by dietary proportion of bermudagrass (Table 4). Ornithine concentration was greatest among bermudagrass levels for 33%, and lysine concentration increased as bermudagrass replaced ryegrass–wheat in the diet.

### 3.5. Experiment 5

Chopped and pelleted bermudagrass was 8.6 and 8.1% CP, and chopped and pelleted ryegrass–wheat was 11.3 and 11.9% CP (DM basis), respectively. Dry matter intake was 0.67, 1.12, 0.92 and 1.37 kg day$^{-1}$ (SE 0.079), digestible N intake was 4.4, 7.0, 8.4 and 14.1 g day$^{-1}$ (SE 0.82) and DE intake was

Table 5
Arterial whole blood amino acid concentrations in sheep consuming bermudagrass and ryegrass–wheat hay coarsely chopped or ground and pelleted (Experiment 5)

| Amino acid | Bermudagrass | | Ryegrass–wheat | | SE | $P$ ($\leq$) | | |
|---|---|---|---|---|---|---|---|---|
| | Coarsely chopped | Ground and pelleted | Coarsely chopped | Ground and pelleted | | Grass source | Grass form | Source × form |
| Aspartate | 29.7 | 26.4 | 39.3 | 28.5 | 3.81 | | 0.09 | |
| Glutamate | 86.2 | 77.8 | 89.4 | 96.7 | 7.14 | | | |
| Asparagine | 68.4 | 74.5 | 81.5 | 83.6 | 10.73 | | | |
| Serine | 73.6 | 93.3 | 93.9 | 125.7 | 9.73 | 0.02 | 0.02 | |
| Glutamine | 338 | 256 | 325 | 269 | 27.2 | | 0.03 | |
| Alanine | 169 | 161 | 157 | 208 | 10.4 | | 0.06 | 0.01 |
| Tyrosine | 49.8 | 67.6 | 71.3 | 97.3 | 8.86 | 0.01 | 0.03 | |
| Ornithine | 109 | 118 | 115 | 134 | 15.6 | | | |
| Histidine | 87.3 | 84.6 | 84.3 | 100.0 | 14.84 | | | |
| Tryptophan | 29.2 | 26.0 | 40.6 | 40.7 | 4.71 | 0.02 | | |
| Valine | 129 | 144 | 170 | 248 | 23.7 | 0.01 | 0.07 | |
| Phenylalanine | 36.0 | 47.3 | 49.8 | 59.2 | 4.98 | 0.02 | 0.06 | |
| Isoleucine | 70.5 | 66.2 | 78.7 | 107.4 | 8.30 | 0.01 | | 0.07 |
| Leucine | 92.9 | 105.7 | 111.2 | 166.5 | 13.59 | 0.01 | | 0.07 |
| Lysine | 132.5 | 117.6 | 113.8 | 77.5 | 11.44 | 0.01 | 0.03 | |

6.0, 9.6, 10.2 and 13.8 MJ $day^{-1}$ (SE 0.91) for chopped bermudagrass, pelleted bermudagrass, chopped ryegrass–wheat and pelleted bermudagrass, respectively (Goetsch et al., 1997b).

Arterial concentrations of most amino acids, including all measured essential amino acids except histidine, were affected by dietary treatments (Table 5). Concentrations of serine ($P = 0.02$), glutamine ($P = 0.03$), tyrosine ($P = 0.03$), valine ($P = 0.07$) and phenylalanine ($P = 0.06$) were greater and concentrations of aspartate ($P = 0.09$), glutamine ($P = 0.03$) and lysine ($P = 0.03$) were lower for pelleted vs. chopped grass. Concentrations of serine ($P = 0.02$), tyrosine ($P = 0.01$) and tryptophan ($P = 0.02$) were greater and lysine concentration was lower ($P = 0.01$) for ryegrass–wheat than for bermudagrass. Interactions between grass source and physical form were observed for concentrations of alanine ($P = 0.01$), isoleucine ($P = 0.07$) and leucine ($P = 0.07$), with grinding and pelleting eliciting greater change in concentrations with ryegrass–wheat than with bermudagrass.

## 4. Discussion

### 4.1. Experiment 1

The increase in arterial concentration of serine as grass quality decreased was not attributable to change in digestible N intake, as indicated by a nonsignificant ($P > 0.10$) correlation. Arterial alanine concentration also numerically increased as grass quality decreased. Typically, the liver removes appreciable alanine and serine for gluconeogenesis (Bergman and Pell, 1984). Perhaps as grass quality and DE intake decreased, demand for glucose metabolism for NADPH production to support fat synthesis decreased, thereby lessening hepatic uptake of glucogenic amino acids.

Factors responsible for greatest concentrations among grasses of valine, isoleucine and leucine for moderate quality grass are unclear. A significant positive correlation between DE intake, as an index of ruminally fermentable OM, and arterial amino acid concentration implies effects of microbial protein synthesis, and a positive relationship with digestible N intake infers influence of forage amino acid intake. Correlations between arterial concentrations of isoleucine and leucine and digestible N and DE intakes were nonsignificant ($P > 0.10$). Conversely, arterial valine concentration was correlated with digestible N ($r = 0.36$; $P = 0.05$) and DE intakes ($r = 0.36$; $P = 0.05$). The effect of digestible N intake as a covariate in the model for valine arterial concentration was significant ($P = 0.04$), and the level of significance of the grass quality effect was lessened as well ($P = 0.06$). Intake of DE as a covariate affected ($P = 0.04$) arterial valine concentration but did not greatly change the significance level of the effect of grass quality ($P = 0.04$). Thus, forage valine intake may have had slightly greater impact than microbial protein synthesis.

The finding that neither grass type nor quality substantially influenced arterial amino acid concentrations probably relates, in part, to the relatively high stage of maturity of these sheep compared with those used in Experiments 4 and 5. Because of presumably low protein accretion by sheep in this experiment, considerable proportions of absorbed amino acids would be used in functions other than protein synthesis including hepatic deamination, regardless of quantities absorbed. Moreover, differences among diets in total feed intake were not marked, apart from low digestible N and DE intakes for the lowest quality temperate grass.

### 4.2. Experiment 2

Effects of alfalfa inclusion and level on arterial alanine concentration did not occur as a result of digestible N intake, as indicated by a nonsignificant ($P > 0.10$) correlation. These treatment effects may relate to differences in peripheral alanine release or hepatic uptake. Hepatic alanine uptake for gluconeogenesis could have decreased with alfalfa inclusion in the diet because of increased propionate availability (Nocek and Tamminga, 1991), which was implicated by a decrease in the acetate to propionate ratio in ruminal fluid and an increase in portal-drained viscera propionate release (Patil et al., 1996).

Factors responsible for the difference between bermudagrass and ryegrass–wheat diets in glutamine concentration in arterial blood are unclear. Glutamine is extensively metabolized by the gut (Kelly et al., 1993); however, arterial glutamine concentra-

tion and energy use by the portal-drained viscera were not correlated ($P > 0.10$). Greater ornithine concentration for 40 vs. 20% alfalfa probably resulted from corresponding differences in digestible N intake and hepatic ammonia uptake and urea release (Bergman and Pell, 1984; Patil et al., 1996). Arterial ornithine concentration was related to hepatic uptake of ammonia ($r = -0.48$; $P = 0.05$) and urea release ($r = 0.54$; $P = 0.02$). Furthermore, urea release by the liver had a significant ($P = 0.03$) effect on arterial ornithine concentration when included in the model as a covariate, which rendered the difference between 20 and 40% alfalfa nonsignificant ($P = 0.19$).

Lysine concentration in arterial blood was not related ($P > 0.10$) to DE or digestible N intakes. The difference in arterial lysine concentration between 20 and 40% dietary alfalfa and relatively little effect of substituting 20% alfalfa for grass may indicate that lysine availability approximated potential for use in protein synthesis with 0 and 20% dietary alfalfa. With 40% alfalfa, lysine availability apparently exceeded potential for incorporation into synthesized protein (Owens and Bergen, 1983), with a greater ratio of lysine oxidation to use in protein synthesis than for 0 or 20% alfalfa implicated. Similar projections apply to other amino acids as well, such as histidine, valine and leucine.

In general, with these nearly mature sheep and ad libitum consumption, it seems that dietary inclusion of 40% alfalfa resulted in essential amino acid availability in excess of potential for use in protein synthesis, with greater coincidence for 0 and 20% alfalfa. These results do not identify one particular amino acid as being considerably more limiting in availability than others, since concentrations of a number of essential amino acids were affected by dietary alfalfa level, and in no instance did arterial amino acid concentration increase as markedly with the first addition of alfalfa as with the second. Hence, it does not appear that degrees to which different amino acids limited protein synthesis were changed by dietary inclusion of 20 vs. 40% alfalfa in these grass-based diets. In part, this finding may relate to simultaneous changes in intakes of DE and of all amino acids, as would occur in many production settings in which mixed grass-legume swards or hay crops are ingested ad libitum.

### 4.3. Experiment 3

Arterial levels of essential amino acids are influenced by interacting factors of amino acid intake and ruminal microbial degradation, DE intake, the profile of all amino acids absorbed and capacity of the animal to utilize amino acids in synthesis of protein. Thus, the absence of differences between bermudagrass and ryegrass–wheat diets in arterial amino acid concentrations is in accordance with only one amino acid arterial concentration difference between these grass sources in Experiment 2 with ad libitum intake.

### 4.4. Experiment 4

The lack of treatment effects on arterial concentrations of most amino acids is in general accordance with previous results with more mature sheep. Significant treatment effects in this experiment occurred only for ornithine and lysine. Ornithine concentration was not correlated with urea release by the liver or with ammonia release by the portal-drained viscera or hepatic uptake, in contrast to relationships noted in Experiment 2. Arterial lysine concentration was negatively correlated with DE ($r = -0.48$; $P = 0.05$) and digestible N intakes ($r = -0.50$; $P = 0.04$). It seems most plausible that as DE intake and energy available to peripheral tissues increased, use of amino acids in peripheral protein synthesis increased, resulting in decreased arterial lysine concentration. The level of significance of the effect of DE intake included in the model as a covariate ($P = 0.06$) was slightly less than that of the difference between DE intake and splanchnic bed energy use ($P = 0.04$), as a relative index of extra-splanchnic tissue energy availability (Goetsch et al., 1997b). Both covariates elicited nonsignificant linear effects of bermudagrass level in the diet, although the $P$ value for DE intake as a covariate was slightly less ($P = 0.15$) than that for the covariate extra-splanchnic tissue energy availability ($P = 0.22$). As ryegrass–wheat in the diet increased and bermudagrass decreased, lysine absorption may have increased less than did the potential for use of lysine in protein synthesis. For other measured essential amino acids, perhaps increased absorption as ryegrass–wheat replaced bermudagrass in the diet more closely coincided with

increased potential for incorporation in synthesized protein, thereby preventing significant change in arterial concentrations.

Overall, these results indicate that the degree to which lysine limited protein synthesis in these growing sheep may have varied with DE intake as influenced by dietary proportions of ryegrass–wheat and bermudagrass, being most limiting when DE intake was greatest with 100% ryegrass–wheat. In support, Gibb et al. (1992) supplemented growing beef calves with different N sources and, based on plasma amino acid concentrations, noted that lysine was most limiting to growth only when BW gain was greatest. Similarly, Goetsch et al. (1997c) suggested that the potential for efficient use of supplemental ruminally undegraded protein sources can be less with tropical vs. temperate grasses because of lower DE intake and less energy available to support peripheral protein synthesis. Also, Owens and Bergen (1983) indicated that it is likely that most limiting amino acids vary with production stages and levels because of shifts in the types of proteins being synthesized. In the present experiment, the greatest tissue shift in proportions of total protein synthesized as DE intake increased with increasing dietary proportion of ryegrass–wheat presumably was from the splanchnic bed to the periphery.

### 4.5. Experiment 5

Factors responsible for the effect of grass form on arterial aspartate concentration are unclear; aspartate concentration was not related to DE or digestible N intakes ($P > 0.10$). Arterial serine concentration correlated with DE ($r = 0.65$; $P = 0.01$) and digestible N intakes ($r = 0.67$; $P < 0.01$). Inclusion of DE intake in the model as a covariate resulted in grass source and physical form $P$ values of 0.15 and 0.12, and those for the model with digestible N intake as a covariate were 0.75 and 0.14, respectively. Thus, differences in serine intake may have been more responsible for treatment effects on arterial serine concentration than differences in microbial protein synthesis.

Glutamine concentration was not related to DE or digestible N intakes ($P > 0.10$). Hence, grass source and form either impacted specific substrates metabolized by the gut, or peripheral muscle glutamine release did not vary simply with changes in DE intake elicited by grass source and form. Relative importance of DE and digestible N intakes to arterial tyrosine concentration cannot be discerned, as both were correlated (DE intake: $r = 0.86$, $P < 0.01$; digestible N intake: $r = 0.84$, $P < 0.01$) and inclusion of each in the model as covariates rendered effects of grass source and form nonsignificant ($P > 0.10$).

Of the six essential amino acids with concentrations affected by dietary treatments, five (i.e., tryptophan, valine, phenylalanine, isoleucine and leucine) can be grouped together based on positive correlations with DE intake and digestible N intakes. Thus, increased arterial concentrations resulted from increased microbial protein synthesis and(or) ruminal outflow of intact grass protein, which presumably accompanied greater intake of ryegrass–wheat than bermudagrass and of pelleted vs. chopped grass. Inclusion of intake of DE or digestible N as a covariate accounted for significant ($P < 0.05$) variability and elicited nonsignificant effects of dietary treatments.

It is unclear why differences in arterial concentrations of essential amino acids other than lysine were observed in this experiment but not in Experiment 4, since grass sources were the same. Differences between the two experiments in DE intake may have been partially responsible for the disparity; DE intake for the chopped bermudagrass diets was 7.6 and 6.0 MJ day$^{-1}$ and that for chopped ryegrass–wheat diets was 12.2 and 10.2 MJ day$^{-1}$ in Experiments 4 and 5, respectively. Perhaps with greater DE intake in Experiment 4, only one treatment effect on an essential amino acid concentration occurred because of greater potential for peripheral protein synthesis and accompanying amino acid incorporation in protein.

Arterial lysine concentration was related to DE intake ($r = -0.48$; $P = 0.06$), digestible N intake ($r = -0.61$; $P = 0.01$) and extra-splanchnic tissue energy availability ($r = -0.56$; $P = 0.03$). These relationships and treatment effects generally correspond with observations in Experiment 4. In Experiment 5, with lowest DE intake and extra-splanchnic tissue energy availability for the chopped bermudagrass diet, lysine availability may have exceeded potential for use in protein synthesis. As DE intake

and extra-splanchnic tissue energy availability were increased by grinding and pelleting of bermudagrass in Experiment 5 or by substitution of ryegrass–wheat for bermudagrass in Experiments 4 and 5, relative changes in energy availability and, thus, in the rate of protein synthesis, may have been greater than in lysine availability, which thereby decreased arterial lysine concentration. Similar findings were noted when DE intake was increased to the highest level by grinding and pelleting of ryegrass–wheat. These effects for lysine differ considerably from those with tryptophan, valine, phenylalanine, isoleucine and leucine. Therefore, increases in protein synthesis facilitated by increased DE intake with use of ryegrass–wheat vs. bermudagrass and(or) grinding and pelleting appeared accompanied by relatively less potential for incorporation in synthesized protein of these amino acids compared with absorption.

## 5. Conclusions

With grass diets consumed ad libitum or in limited quantities by wethers near maturity, differences in grass source and(or) quality generally did not markedly affect arterial concentrations of essential amino acids. Likewise, with ad libitum intake grass source did not alter effects of dietary alfalfa level on arterial amino acid concentrations. In general, it appeared that dietary inclusion of 40% alfalfa resulted in excessive essential amino acid availability relative to potential for use in protein synthesis, with greater coincidence noted for 0 and 20% alfalfa.

With growing wethers, increasing feed intake by substituting ryegrass–wheat for bermudagrass or by grinding and pelleting decreased arterial lysine concentration, suggesting that changes in potential lysine utilization in protein synthesis were greater than in lysine absorption and subsequent availability. Conversely, generally opposite shifts with differences in grass source and physical form were noted for tryptophan, valine, phenylalanine, isoleucine and leucine. Thus, lysine absorption with grass hay diets for which DE intake is relatively high may be inadequate for maximal protein synthesis by growing wethers but sufficient with lower quality grass.

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## References

ARC, 1980. The Nutrient Requirements of Ruminant Livestock. Agricultural Research Council, Commonwealth Agricultural Bureaux, Farnham Royal Slough, England, pp. 121–181.

Bergman, E.N., Pell, J.M., 1984. Integration of amino acid metabolism in the ruminant. In: Gilchrist, F.M.C., Mackie, R.I. (Eds.), Herbivore Nutrition in the Subtropics and Tropics. The Science Press, Craighall, South Africa, pp. 613–630.

Egan, A.R., Rogers, Q.R., 1978. Amino acid imbalance in ruminant lambs. Aust. J. Agric. Res. 29, 1263–1279.

Fenderson, C.L., Bergen, W.G., 1975. An assessment of essential amino acid requirements of growing steers. J. Anim. Sci. 41, 1759–1766.

Gibb, D.J., Klopfenstein, T.J., Britton, R.A., Lewis, A.J., 1992. Plasma amino acid response to graded levels of escape protein. J. Anim. Sci. 70, 2885–2892.

Goetsch, A.L., Patil, A.R., Galloway, D.L. Sr., Kouakou, B., Wang, Z.S., Park, K.K., Rossi, J.E., 1997a. Net flux of nutrients across splanchnic tissues in wethers consuming ad libitum different grass sources and physical forms. Br. J. Nutr. 77, 769–781.

Goetsch, A.L., Patil, A.R., Galloway, D.L. Sr., Wang, Z.S., Kouakou, B., Park, K.K., Rossi, J.E., 1997b. Oxygen consumption by splanchnic tissues in wethers consuming ad libitum different proportions of bermudagrass and ryegrass–wheat. Arch. Anim. Nutr. 50, 1–11.

Goetsch, A.L., Patil, A.R., Wang, Z.S., Park, K.K., Galloway Sr., D.L., Rossi, J.E., Koukou, B., 1997c. Net fluxof nutrients across splanchnic tissues in wethers consuming bermidagrass or ryegrass–wheat hay supplemented with rumen undegradable protein. Small Rum. Res., 25: 119–128.

Harmon, D.L., Gross, K.L., Kreikemeier, K.K., Coffey, K.P., Avery, T.B., Klindt, J., 1991. Effects of feeding endophyte-infected fescue hay on portal and hepatic nutrient flux in steers. J. Anim. Sci. 69, 1223–1231.

Kelly, J.M., Park, H., Summers, M., Milligan, L.P., 1993. Interactions between protein and energy metabolism. In: Forbes,

J.M., France, J. (Eds.), Quantitative Aspects of Ruminant Digestion and Metabolism. C.A.B. International, Oxon, UK, pp. 341–362.

Minson, D.J., 1990. Forage in Ruminant Nutrition. Academic Press, San Diego, CA, pp. 9–149.

Nimrick, K., Hatfield, E.E., Kaminski, J., Owens, F.N., 1970a. Qualitative assessment of supplemental amino acid needs for growing lambs fed urea as the sole nitrogen source. J. Nutr. 100, 1293–1300.

Nimrick, K., Hatfield, E.E., Kaminski, J., Owens, F.N., 1970b. Quantitative assessment of supplemental amino acid needs for growing lambs fed urea as the sole nitrogen source. J. Nutr. 100, 1301–1306.

Nocek, J.E., Tamminga, S., 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. J. Dairy Sci. 74, 3598–3629.

Owens, F.N., Bergen, W.G., 1983. Nitrogen metabolism of ruminant animals: historical perspective, current understanding and future implications. J. Anim. Sci. 57 (Suppl. 2), 498–518.

Patil, A.R., Goetsch, A.L., Park, K.K., Kouakou, B., Galloway, D.L. Sr., Johnson, Z.B., 1995a. Influences of grass source on net flux of nutrients across splanchnic tissues in sheep with restricted intake. Arch. Anim. Nutr. 48, 257–269.

Patil, A.R., Goetsch, A.L., Park, K.K., Kouakou, B., Galloway, D.L. Sr., Johnson, Z.B., 1996. Influences of grass source and legume level on net flux of nutrients across splanchnic tissues in sheep. Small Rum. Res. 22, 111–122.

Patil, A.R., Goetsch, A.L., Park, K.K., Kouakou, B., Galloway, D.L. Sr., West, C.P., Johnson, Z.B., 1995b. Net flux of nutrients across splanchnic tissues in sheep fed tropical vs. temperate grass hay of moderate or low qualities. Livest. Prod. Sci. 43, 49–61.

Rainin, 1990. AAAnalysis, Type O High Performance Reversed-Phase Columns: Instruction Manual. Rainin Instrument, Woburn, MA, PI-63, 9920-073.

SAS, 1990. SAS/STAT User's Guide, Version 6, 4th edn., Vol. 2. Statistical Analysis System Inst., Cary, NC, pp. 891–996.